

Culture Confirmation-Further Identification – *Bacillus anthracis*

Purpose

To provide guidelines on the transport of gram positive, nonmotile, nonhemolytic bacilli to the OPH Central Laboratory for testing

Policy

The OPH Central Laboratory will only accept pure cultures of gram positive, nonmotile, nonhemolytic bacilli for 'Rule out Anthrax' testing.

Specimen Collection, Handling, Storage

1. The causative agent of Anthrax, is a rod-shaped, gram positive, sporulating bacterium with the spores constituting the usual infective form.
2. Anthrax presents as three distinct clinical syndromes: cutaneous, inhalational and gastrointestinal. The cutaneous form (also referred to as malignant pustule) occurs most frequently on the hands and forearms of persons working with infected livestock. It begins with a papule followed by formation of a blister-like fluid-filled vesicle. The vesicle typically dries and forms a coal-black scab, hence the term Anthrax (Greek for coal). Sometimes this local infection will develop into a systemic infection which is often fatal. Endemic inhalational Anthrax, known as Woolsorters' Disease, is a rare infection contracted by the inhalation of spores of *B. anthracis*. Gastrointestinal Anthrax is caused by the ingestion of insufficiently cooked meat from infected animals. In man, the mortality of untreated cutaneous Anthrax ranges up to 25%; in inhalational and gastrointestinal cases, the case fatality rate is almost 100%.
3. Acceptable specimens: Pure culture of gram positive bacilli, non-motile, non-hemolytic, that a Level A laboratory is unable to rule out as *Bacillus anthracis*, grown on solid or in liquid media.
4. If patient's symptoms are indicative of *Bacillus anthracis* infection and you are unable to rule out *Bacillus anthracis* in the laboratory or if you receive multiple patients with the same unknown organism please contact the State Epidemiologist at 504-568-5005 and the OPH Central Laboratory Molecular Bacteriology Unit at 504-568-2373 or General Bacteriology Unit at 504-568-7683 prior to shipment.

Procedure

Step	Action
1	Fill out Lab Form 93 with subject's name, sex, age, home address, date of specimen collection, source of isolation and submitter information.
2	Under organism suspected on the Lab Form 93 write Rule out Anthrax
3	Attach the corresponding Lab Form 93 identification tag to sample container. If there is more than one sample container for any given patient, submit one Lab Form 93 per specimen.
4	Incubate the culture for 18-24 hours at 35C in ambient air.
5	Ship incubated culture as an infectious specimen using the provided Infectious Substance mailers. Mailers can be obtained by contacting Bonnie Fiorito (504-568-5443) or Wayne Dupree (504-568-3453) and completing and faxing the Request for Specimen Shipping Supplies form (see Appendix 5). Include Lab Form 93 in the Infectious Substance Mailer. Ship to the OPH Central Laboratory, New Orleans. Do not ship plates through the mail. Plates need to be hand delivered by a courier.

Culture Confirmation-Further Identification – *Brucella* Species

Purpose

To provide guidelines on the transport of gram negative coccobacilli to the OPH Central Laboratory for testing

Policy

The OPH Central Laboratory will only accept pure cultures of gram negative coccobacilli that a Level A laboratory is unable to rule out as *Brucella* spp.

The OPH Central Laboratory does not test serum titers for total antibody and IgG to *Brucella* spp. Serum samples sent to the OPH Central Laboratory for *Brucella* microagglutination testing will be sent to the CDC for further testing.

Specimen Collection, Handling, Storage

1. Brucellosis may present as nonspecific febrile illness which resembles influenzae. Fever, headache, myalgia, arthralgia, back pain, sweats, chills, generalized weakness and malaise are common complaints. Cough and pleuritic chest pain may occur in up to 20% of cases, but these are usually not associated with acute pneumonitis. Pulmonary symptoms may not correlate with radiographic findings. The chest x-ray may be normal, or show lung abscesses, single or military nodules, bronchopneumonia, enlarged hilar lymph nodes and pleural effusions. Gastrointestinal symptoms occur in up to 70% of adult cases and less frequently in children. These include anorexia, nausea, vomiting, diarrhea or constipation. Ileitis, colitis and granulomatous or a mononuclear infiltrative hepatitis may occur. Lumbar pain and tenderness can occur in up to 60% of cases and is due to various osteoarticular infections of the skeletal system.
2. Paravertebral abscesses may occur and can be imaged by CT scan or MRI. CT scans often show vertebral sclerosis. Vertebral and disc space destruction may occur in chronic cases. One or, less frequently, both sacroiliac joints may be infected causing low back and buttock pain that is intensified by stressing the sacroiliac joints on physical exam.. Hepatomegaly and splenomegaly can occur in up to 45-63% of cases. Peripheral joint involvement may vary from pain on range of motion testing to joint immobility and effusion. Peripheral joint effusions usually show mononuclear cell predominance and organisms can be isolated in up to 50% of cases. The hip joints are the most commonly involved peripheral, but ankle, knee and sternoclavicular joint infection may occur. Plain radiographs of involved sacroiliac joints usually show blurring of articular margins and widening of the joint space. Technetium or Gallium-67 bone scans are 90% sensitive for detecting sacroileitis and will

also detect other sites of bone and joint involvement; they are also useful for differentiating sacroiliac from hip joint involvement.

3. Acceptable specimens: Pure culture of gram negative coccobacilli that a Level A laboratory is unable to rule out as *Brucella* spp., grown on solid or in liquid media. Blood slant (5% sheep blood) or Nutrient Agar slant is recommended. Serum samples sent to the OPH Central Laboratory for *Brucella* microagglutination testing will be sent to the CDC for further testing.
4. If patient's symptoms are indicative of *Brucella* spp. infection and you are unable to rule out *Brucella* in the laboratory or if you receive multiple patients with the same unknown organism please contact the State Epidemiologist at 504-568-5005 and the OPH Central Laboratory Molecular Bacteriology Unit at 504-568-2373 or General Bacteriology Unit at 504-568-7683 prior to shipment.

Special safety precautions

Brucellosis is one of the most commonly reported laboratory-acquired infections. All materials suspected of containing *Brucella* spp. should be handled with gloves in a Class II biological safety cabinet. Decontamination can be achieved by wiping down contaminated surfaces with a freshly-made (less than 7 days old) 1:10 aqueous solution of household bleach.

Procedure

Step	Action
1	Fill out Lab Form 93 with subject's name, sex, age, home address, date of specimen collection, source of isolation and submitter information.
2	Under organism suspected on the Lab Form 93 write Rule Out Brucella.
3	Attach the corresponding Lab Form 93 identification tag to sample container. If there is more than one sample container for any given patient, submit one Lab Form 93 per specimen.
4	Incubate culture for 18-24 hours before shipping to the OPH Central Laboratory.
5	Ship incubated culture as an infectious specimen using the provided Infectious Substance mailers. Mailers can be obtained by contacting Bonnie Fiorito (504-568-5443) or Wayne Dupree (504-568-3453) and completing and faxing the Request for Specimen Shipping Supplies form (see Appendix 5). Include Lab Form 93 in the Infectious Substance Mailer. Ship to the OPH Central Laboratory, New Orleans. Do not ship plates through the mail. Plates need to be hand delivered by a courier.

Culture Confirmation-Further Identification – *Burkholderia mallei*

Purpose

To provide guidelines on the collection and transport of suspected *Burkholderia mallei* samples to the OPH Central Laboratory for testing

Policy

The OPH Central Laboratory will only accept pure cultures of gram negative coccobacilli that a Level A laboratory is unable to rule out as *Burkholderia mallei*.

Specimen Collection, Handling, Storage

1. *Burkholderia mallei* is the etiologic agent of Glanders. Glanders may occasionally be transmitted to humans and other animals by direct contact with infected animals or pure cultures. *B. mallei* causes necrosis of the tracheobronchial tree, pustular skin lesions, febrile pneumonia or sepsis, and abscesses depending upon the mode of infection. Characteristic local manifestations resulting from skin or mucosal contact may include wound infection and ulceration, lymphatic drainage (farcy buds), and abscesses in any site, particularly, muscle and subcutaneous tissue. A widespread necrotizing skin eruption has been described as a terminal feature in patients with disseminated Glanders. Glanders can have a chronic form with multiple abscesses of liver and spleen, months and years after exposure.
2. Acceptable specimens: Pure culture of small, aerobic, non-motile, gram negative coccobacilli that a Level A laboratory is unable to rule out as *Burkholderia mallei*, grown on solid or in liquid media. Blood slant or Nutrient Agar slant is recommended.
3. If patient's symptoms are indicative of *Burkholderia mallei* infection and you are unable to rule out *Burkholderia mallei* in the laboratory or if you receive multiple patients with the same unknown organism please contact the State Epidemiologist at 504-568-5005 and the OPH Central Laboratory Molecular Bacteriology Unit at 504-568-2373 or General Bacteriology Unit at 504-568-7683 prior to shipment.

Procedure

Step	Action
1	Fill out Lab Form 93 with subject's name, sex, age, home address, date of specimen collection, source of isolation and submitter information.
2	Under organism suspected on the Lab Form 93 write Rule Out <i>Burkholderia mallei</i> or Rule out Glanders.

3	Attach the corresponding Lab Form 93 identification tag to sample container. If there is more than one sample container for any given patient, submit one Lab Form 93 per specimen.
4	Incubate culture for 18-24 hours before shipping to the OPH Central Laboratory.
5	Ship incubated culture as an infectious specimen using the provided Infectious Substance mailers. Mailers can be obtained by contacting Bonnie Fiorito (504-568-5443) or Wayne Dupree (504-568-3453) and completing and faxing the Request for Specimen Shipping Supplies form (see Appendix 5). Include Lab Form 93 in the Infectious Substance Mailer. Ship to the OPH Central Laboratory, New Orleans. Do not ship plates through the mail. Plates need to be hand delivered by a courier.

Culture Confirmation-Further Identification – *Burkholderia pseudomallei*

Purpose

To provide guidelines on the collection and transport of suspected *Burkholderia pseudomallei* samples to the OPH Central Laboratory for testing

Policy

The OPH Central Laboratory will only accept pure cultures of gram negative bacilli that a Level A laboratory is unable to rule out as *Burkholderia pseudomallei*.

Specimen Collection, Handling, Storage

1. *Burkholderia pseudomallei* is the etiologic agent of Melioidosis. The clinical presentation of *B. pseudomallei* infection is very broad and includes mild or subclinical infections, mostly among children, as well as latent infections in people residing in endemic areas (Southeast Asia and Australia). Most of these infections are subclinical and severe infections are rare.
2. Two common clinical presentations are ‘septicemia melioidosis’ (also known as community acquired ‘sepsis syndrome’), and ‘localized melioidosis’ (lung, parotid gland, etc.). Symptoms in patients with sepsis syndrome include high fever, rigors, confusion, stupor, jaundice, diarrhea, leucocytosis, coagulopathy, evidence of renal and hepatic impairment, and abnormal chest x-ray (nodular shadowing). These clinical signs and symptoms are found in 60-80% of patients; mortality is about 20% in localized infections, but approaches 95% following septic shock and many patients die within 48 hours of hospital admission. Cases of localized melioidosis present with cutaneous and/or subcutaneous abscesses and lymphadenitis, which can progress to osteomyelitis, cystitis, keratitis, and brain abscesses. Localized melioidosis also frequently occurs in the lung with abscess(es) and profound weight loss, which may be confused with tuberculosis.
3. Acceptable specimens: Pure culture of medium, aerobic, motile, gram negative slender rods with bipolar staining that a Level A laboratory is unable to rule out as *Burkholderia pseudomallei*, grown on solid or in liquid media. Blood slant or Nutrient Agar slant is recommended.
4. If patient’s symptoms are indicative of *Burkholderia pseudomallei* infection and you are unable to rule out *Burkholderia pseudomallei* in the laboratory or if you receive multiple patients with the same unknown organism please contact the State Epidemiologist at 504-568-5005 and the

OPH Central Laboratory Molecular Bacteriology Unit at 504-568-2373 or General Bacteriology Unit at 504-568-7683 prior to shipment.

Procedure

Step	Action
1	Fill out Lab Form 93 with subject's name, sex, age, home address, date of specimen collection, source of isolation and submitter information.
2	Under organism suspected on the Lab Form 93 write Rule Out <i>Burkholderia pseudomallei</i> or Rule Out Melioidosis.
3	Attach the corresponding Lab Form 93 identification tag to sample container. If there is more than one sample container for any given patient, submit one Lab Form 93 per specimen.
4	Incubate culture for 18-24 hours before shipping to the OPH Central Laboratory.
5	Ship incubated culture as an infectious specimen using the provided Infectious Substance mailers. Mailers can be obtained by contacting Bonnie Fiorito (504-568-5443) or Wayne Dupree (504-568-3453) and completing and faxing the Request for Specimen Shipping Supplies form (see Appendix 5). Include Lab Form 93 in the Infectious Substance Mailer. Ship to the OPH Central Laboratory, New Orleans. Do not ship plates through the mail. Plates need to be hand delivered by a courier.

Culture Confirmation-Further Identification – *Clostridium botulinum*/Botulinum Toxin

Purpose

To provide guidelines on the transport of suspected *Clostridium botulinum* samples to the OPH Central Laboratory for routing to CDC

Policy

The OPH Central Laboratory does not test for *Clostridium botulinum*. All samples are forwarded to CDC for testing.

A case of botulinum is defined as an illness characterized by clinical manifestations relating to the nervous system (ptosis, blurred or double vision, dry mouth and sore throat are usually the first symptoms followed by descending paralysis) that is laboratory confirmed.

Specimen Collection, Handling, Storage

1. A CDC Form 50 providing patient history must be completed for each specimen. The OPH Central Laboratory will forward the paperwork and specimens to CDC.
2. Acceptable specimens: 15cc serum (2cc minimum)
 25g stool
 Suspect food leftovers, if available
3. Whole stool and serum should be shipped refrigerated. Frozen food should be shipped frozen. Refrigerated and room temperature food should be sent refrigerated.
4. **The State Epidemiologist MUST BE contacted (504-568-5005) prior to shipment of any potential *C. botulinum* specimens. The specimen will only be shipped at the request of the State Epidemiologist. If the specimen is approved for shipment by the State Epidemiologist, contact the OPH Central Laboratory Molecular Bacteriology Unit at 504-568-2373 or General Bacteriology Unit at 504-568-7683 prior to shipment.**

Procedure

Step	Action
1	Fill out Lab Form 93 with subject's name, sex, age, home address, date of specimen collection, source of isolation and submitter information. Lab Form 47 should be filled out for food. Information on Lab Form 47 should include name of collector, point of collection, type of food collected,

	condition food was found, manufacturer, lot number and requested test (C. botulinum).
2	Under organism suspected on the Lab Form 93 write C. botulinum.
3	Attach the corresponding Lab Form 93/Lab Form 47 identification tag to sample container. If there is more than one sample container for any given patient, submit one Lab Form 93/Lab Form 47 per specimen.
4	For each sample, completely fill out a CDC Form 50 (see Appendix 6 for sample form). You may request a faxed copy of the CDC Form 50 from the OPH Central Laboratory.
5	Ship samples to the OPH Central Laboratory as diagnostic specimens using the provided Diagnostic Specimen mailers. Mailers can be obtained by contacting Bonnie Fiorito (504-568-5443) or Wayne Dupree (504-568-3453) and completing and faxing the Request for Specimen Shipping Supplies form (see Appendix 5). Include Lab Form 93/Lab Form 47 and CDC History Form. Call the OPH Central Laboratory with approximate delivery date/time.

Culture Confirmation-Further Identification – *Francisella tularensis*

Purpose

To provide guidelines on the collection and transport of suspected *Francisella tularensis* samples to the OPH Central Laboratory for testing

Policy

The OPH Central Laboratory will only accept pure cultures of gram negative coccobacilli that a Level A laboratory is unable to rule out as *Francisella tularensis*.

Specimen Collection, Handling, Storage

1. Tularemia is a zoonotic disease that humans typically acquire after contact with tissues or bodily fluids of infected animals, or from bites of infected deerflies, mosquitoes, or ticks. The disease remains widely enzootic in North America, Europe, and northern Asia. Human cases are usually sporadic, but outbreaks do occur.
2. Tularemia presents in humans primarily as an ulceroglandular disease (45-80% of reported cases), as glandular infection (10-25%) and, less frequently (<5%), as oculoglandular, septic, oropharyngeal, typhoidal and pneumonic forms. Onset is sudden, and symptoms include fever, chills, headache, generalized body aches (often prominent in the lower back), coryza, pharyngitis, cough, and chest pain/tightness. Without treatment, nonspecific symptoms usually persist for several weeks. Sweats, chills, progressive weakness, and weight loss characterize the illness. Any of the principal forms of tularemia may be complicated by bacteremic spread, leading to tularemic pneumonia, sepsis, and meningitis.
3. Acceptable specimens: Pure culture of small, aerobic, non-motile, gram negative coccobacilli that a Level A laboratory is unable to rule out as *Francisella tularensis*, grown on solid or in liquid media. Blood slant, Nutrient Agar slant or cysteine-supplemented agar slant (BCYE, Cysteine-heart) is recommended.
4. If patient's symptoms are indicative of *Francisella tularensis* infection and you are unable to rule out *Francisella tularensis* in the laboratory or if you receive multiple patients with the same unknown organism please contact the State Epidemiologist at 504-568-5005 and the OPH Central Laboratory Molecular Bacteriology Unit at 504-568-2373 or General Bacteriology Unit at 504-568-7683 prior to shipment.

Procedure

Step	Action
1	Fill out Lab Form 93 with subject's name, sex, age, home address, date of specimen collection, source of isolation and submitter information.
2	Under organism suspected on the Lab Form 93 write Rule Out Tularemia.
3	Attach the corresponding Lab Form 93 identification tag to sample container. If there is more than one sample container for any given patient, submit one Lab Form 93 per specimen.
4	Incubate culture for 18-24 hours before shipping to the OPH Central Laboratory.
5	Ship incubated culture as an infectious specimen using the provided Infectious Substance mailers. Mailers can be obtained by contacting Bonnie Fiorito (504-568-5443) or Wayne Dupree (504-568-3453) and completing and faxing the Request for Specimen Shipping Supplies form (see Appendix 5). Include Lab Form 93 in the Infectious Substance Mailer. Ship to the OPH Central Laboratory, New Orleans. Do not ship plates through the mail. Plates need to be hand delivered by a courier.

Culture Confirmation-Further Identification – *Yersinia pestis*

Purpose

To provide guidelines on the collection and transport of suspected *Yersinia pestis* samples to the OPH Central Laboratory for testing

Policy

The OPH Central Laboratory will only accept pure cultures of gram negative bacilli that a Level A laboratory is unable to rule out as *Yersinia pestis*.

Specimen Collection, Handling, Storage

1. Plague is a zoonotic disease that humans typically acquire from the bite of infected fleas, from direct contact with contaminated tissue, and by inhalation of bacteria-laden droplets.
2. Plague presents in two forms: Bubonic and Pneumonic. Bubonic plague is the most common form of infection. Host macrophages in the lymph nodes take up the bacteria, causing the lymph node to become inflamed (bubo), enlarged, and painful as the bacteria replicate. From the infected lymph node, bacteria sometimes become blood-borne and occasionally lodge in the lungs, resulting in Pneumonic plague. When plague infection becomes pneumonic, direct person-to-person transmission via bacterial aerosolization becomes a realistic threat. Progression of pneumonic plague is rapid, and if untreated, may lead to death in a few days.
3. Acceptable specimens: Pure culture of small, non-motile, gram negative bacilli that a Level A laboratory is unable to rule out as *Yersinia pestis*, grown on solid or in liquid media. Blood slant or Nutrient Agar slant.
4. If patient's symptoms are indicative of *Yersinia pestis* infection and you are unable to rule out *Yersinia pestis* in the laboratory or if you receive multiple patients with the same unknown organism please contact the State Epidemiologist at 504-568-5005 and the OPH Central Laboratory Molecular Bacteriology Unit at 504-568-2373 or General Bacteriology Unit at 504-568-7683 prior to shipment.

Procedure

Step	Action
1	Fill out Lab Form 93 with subject's name, sex, age, home address, date of specimen collection, source of isolation and submitter information.
2	Under organism suspected on the Lab Form 93 write Rule Out <i>Yersinia pestis</i> .

3	Attach the corresponding Lab Form 93 identification tag to sample container. If there is more than one sample container for any given patient, submit one Lab Form 93 per specimen.
4	Incubate culture for 18-24 hours before shipping to the OPH Central Laboratory.
5	Ship incubated culture as an infectious specimen using the provided Infectious Substance mailers. Mailers can be obtained by contacting Bonnie Fiorito (504-568-5443) or Wayne Dupree (504-568-3453) and completing and faxing the Request for Specimen Shipping Supplies form (see Appendix 5). Include Lab Form 93 in the Infectious Substance Mailer. Ship to the OPH Central Laboratory, New Orleans. Do not ship plates through the mail. Plates need to be hand delivered by a courier.